Title of thesis:

STUDY ON EFFECT OF SELECTED ORGANOCHLORINE PESTICIDES ON BIOCHEMICAL, REPRODUCTIVE, HAEMATOLOGICAL AND RESPIRATORY ASPECTS OF *CYPRINUS CARPIO* (LINNAEUS, 1758)

Synopsis of the Ph.D work Submitted To

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INTRODUCTION

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) integrate the organochlorine compounds group (OCCs). They are responsible of relevant environmental contamination representing potential risks to both, human health and the environment. Although, organochlorine like Dieldrin and Methoxychlor have been worldwide used for industrial and agricultural purpose, respectively, most of them are currently forbidden with the exception of Endosulfan pesticide (Masuda *et al.*, 2012). Their physicochemical properties, such as molecular weight, shape, hydrophobicity, vapor pressure and environmental persistence define their behavior and environmental bioavailability. Hence, OCPs could reach to non-target aquatic environments where they are preferentially sorbed onto dissolved and particulate organic matter or accumulated by biota.Organochlorine compounds have been found to have toxic effects on aquatic organisms (Zhou *et al.*,1999).

To know the toxicity of organochlorine compounds (OCCs) and polychlorinated biphenyls (PCBs) in 845 stream sites across United States, 5 principal components have been identified that account for 77% of total variance of OCCs. These were Chlordane related compounds and Dieldrin .Organochlorines could enter an organisms' body across the skin, from the lungs and could also be absorbed from the gut wall. Cyclodienes, hexachlorocyclohexane, endosulfan and lindane can easily pass through the skin, while the absorption is less in case of dicofol, toxaphene, DDT, mirex and methoxychlor (Echobichon et al.,1996)

Hydrophobic pollutant assessment in aquatic environment could be done using the potential of biota to accumulate these contaminants in the lipid fraction. Fish are particularly useful because they have a big interaction with water, sediments and macrophytes occupying different habitats and trophic levels. Toxicity of OCPs to non-target animals have reported thoroughly and fishes are extremely sensitive to

Dieldrin and Methoxychlor and mortality has been reported a number of time as a result of the pesticide leakage into river (Swackhamer *et al.*, 1988). OCPs are a central nervous system (CNS) poison and have gained significance as a potential environmental pollutant due to its wide spread use in agriculture (Dhananjayan *et al.*, 2010). The common carp, *Cyprinus carpio* is a very important fish generally found in rivers, ponds, and reservoirs. Dieldrin and Methoxychlor are extensively used organochlorine insecticide for agricultural crops (Kannan *et al.*, 1995). Hence the current study was undertaken to evaluate the aquatic toxicity of Dieldrin and Methoxychlor with special emphasis on biochemical, reproductive, haematological and respiratory performance of the carp exposed to sublethal concentrations of commercial grade Dieldrin and Methoxychlor.

Review of literature and development in the subject

(Previous work done in the relevant area):

Saha and Kaviraj (2008) and Datta (2003) reported that, the toxicity of Dieldrin and Methoxychlor pesticides depends upon their structure, stereochemistry and formulations.

Bordajandi et al., (2003), observed significant reduction in feeding rates and body weight of *Cyprinus carpio* exposed to sub-lethal concentrations of organochlorine pesticides.

Masuda et al., (2000), suggested that decrease in WBC count could be the result of autolysis caused due to haemolytic enzymes leaked out by the cells under toxicant stress in organochlorine pesticide treated fishes.

Dhananjayan et al., (2010), Studied histopathological changes in the intestine of *Cyprinus carpio* under aldrin, dieldrin, BHC and DOT toxicity stress. Beyer et al., (201; Bordajandi et al., (2003); Anderson and Weber, (1975); Aly & Azhar, (2013) and Malik et al., (2007), Studied the acute toxicity of Dieldrin and Methoxychlor in fresh water fishes and its effect on oxygen consumption

and observed that the rate of oxygen consumption fluctuated with an increase in the exposure period.

Objectives Of Research/ Proposed Hypothesis

There are gaps in our knowledge of the methodologies for analysis, exposures and effects of the organochlorine pesticides. Therefore, new information and activities relevant to these substances are rapidly emerging. This effort calls for effective coordination of research, testing, assessment and sound management of the substances.

Further work is needed in a number of areas to help us gain better insight into the effects of organochlorides on wildlife populations and to develop and validate novel whole organism assays for interference in vertebrates like fish, amphibians, reptiles and birds, to assess hazards to those species and to evaluate long term environmental effects.

Studies are required where organochlorine pesticide effects are suspected; these should involve a broadly based screen including assessment of gonadal function, behavioral patterns and offspring sex ratio, population size and survival rate. Environmental toxicity studies need to focus on aquatic organisms as exposure to industrial chemicals generally involves discharges to water bodies. Exposure assessment studies like this one provide with the toxicity data required for conservation of organisms likely to be exposed and management of the chemicals involved.

The present study was proposed to evaluate the toxic effect of Dieldrin and Methoxychlor on biochemical, and histological status of Cyprinus carpio and the possible reproductive, haematolgicial and respiratory effects of these selected pesticides in different key organs such as gills, brain and liver of the fishes. The characteristic influence of Dieldrin and Methoxychlor on biochemical, histological, reproductive and hematological aspects may improve the understanding of toxic effects at the sub-cellular level.

About *Cyprinus carpio* (Linnaeus, 1758)

Cyprinus carpio is a fish inhabiting warm, deep, slow flowing and still waters such as lowland rivers and large well vegetated lakes. Introduces in all types of water bodies. This fish spawns along shore in back waters. Larval development occurs in very warm waters, along shallow submerged vegetation. It is omnivorous feeding on a diet of water plants but prefer to scavenge the bottom for insects, crustaceans (zooplankton), cray fish, benthonic worms. It can grow to a maximum length of 120 cm (Murdy et al., 1997). Members of genus Cyprinus are widely consumed in lower and middle class economic group. Now it is contributed much more to the fishery economy in India as compared to china.

The present work proposes to study the effects of selected organochlorine pesticides – Dieldrin and Methoxychlor – on general biology of *Cyprinus carpio.*

About Dieldrin

Dieldrin's former production and use as an insecticide resulted in its direct release to the environment. Dieldrin is also a degradation product of the insecticide aldrin, and the former use of aldrin has contributed to the occurrence of dieldrin in the environment. Dieldrin will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase dieldrin will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals (Jorgenson, 2001). The half-life for the reaction with hydroxyl radicals in air is estimated to be 42 hours. Dieldrin also undergoes direct photolysis in the environment yielding photo dieldrin as the primary degradation product. Particulate-phase dieldrin will be removed from the atmosphere by wet and dry deposition. If released to soil, dieldrin is expected to have low to no mobility based upon Koc values of 1,957 to 23,310 measured in soil and sediment. Dieldrin degrades slowly in soil surfaces with a reported half life of about 7 years in field studies (Beyer et al., 2013). If released into water, dieldrin is expected to adsorb to suspended solids and sediment in water

based upon the Koc data. The hydrolysis half-life of dieldrin has been reported as greater than 4 years. BCF values of 3,300 to 14,500, measured in fish, suggest bioconcentration in aquatic organisms is very high. Although dieldrin is no longer registered for agricultural use in the US, the general population continues to be exposed to this compound due to its long persistence time. Oral ingestion of food is the primary source of exposure for the general population (Bordajandi et al., 2003). Ingestion of contaminated drinking water, inhalation of contaminated air and dermal contact with contaminated soil surfaces are also possible routes of human exposure. When Cyprinu scarpio were introduced to the effluent added water, they get excited and were swimming rapidly with random movements. While in control, fish were swimming with normal movement. Similar results were reported earlier by Anderson and Weber (1975) when guppies were exposed to dieldrin.

About Methoxychlor

Methoxychlor, also known as DMDT, is a manufactured chemical used in the United States for controlling insects. Methoxychlor is effective against flies, mosquitos, cockroaches, and a wide variety of other insects. This insecticide is used on agricultural crops and livestock, and in animal feed, barns, and grain storage bins. Some pesticide products that contain methoxychlor are used for controlling insects in gardens or on pets.

Pure methoxychlor is a pale-yellow powder that has a slightly fruity or musty odor. It does not readily evaporate into air or dissolve in water. Pesticide workers usually dissolve methoxychlor in a petroleum-based liquid and apply it as a spray, or they mix it with other chemicals and apply it as a dust. Application of methoxychlor as an insect killer accounts for most of the methoxychlor that enters the environment (Aly et al., 2013). Since the use of methoxychlor is highly seasonal, the amount that is released to the environment tends to be greater during periods of insect control (spring and summer). Some methoxychlor is released to the environment from chemical

manufacturing plants that make methoxychlor or from manufacturing sites that formulate products containing methoxychlor. A small amount may also be released from hazardous waste sites where it has been disposed of (Malik et al., 2007).

Determination of Total Fecundity would be done made by extracting the ovary from the fish. Analysis of secondary sexual characters like coloration, mating behavior would be done. These experiments would also be performed at different exposure times to the selected chemicals and their effects evaluated in the context of environmental and reproductive heath of the fish.

Methodology to be adopted

The Cyprinus carpio freshwater fish were selected as the test species for the present investigation. Studies will be carried out to assess the acute toxicity at various concentrations of Dieldrin and Methoxychlor. Selection of lethal and sub-lethal concentrations will be done to assess the toxicity level.

The experimental fishes will be divided in to three (3) groups of 20 fishes each. Group I fishes will be control fishes, group II fishes will be treated with Dieldrin and Group III fishes will be treated with Methoxychlor and maintained up to the stipulated period (21 days).

Biochemical changes like protein, carbohydrate, lipids and nucleic acids in the gills, liver and brain of Cyprinus carpio will be studied after 7, 14 and 21 day of exposure.

Hematological parameters like blood markers enzymes, hemoglobin, RBC, WBC, PCV, hematocrit levels will be measured in dieldrin and Methoxychlor exposed fishes.

Effect of Dieldrin and Methoxychlor on histopathological changes in the gills, liver and brain of *Cyprinus carpio* will be studies after 7, 14 and 21 days of exposure.

To assess respiratory changes, oxygen consumption of the fish will be evaluated.

To assess the reproductive changes in fishes, after 24 hours exposure the selected pesticides, GSI, ova diameter, overy weight and acetylcholinesterase activity in ovary and brain of the fish will be studied.

Importance of Study / Society Application

Cyprinus carpio is fish inhabiting deep, slow flowing and still waters. Introduces in all types of water bodies. It is an economically important fish consumed by different strata of the society. Study on biological aspects will be helpful because the fish has good demand and market value. The fisheries sector provides employment. Fish have great significance for man, being an important natural source of protein as well as economic sustenance to many nations. Members of genus Cyprinus are widely consumed in lower and middle class economic group. Now it is contributing to the fishery economy in India. Its wide consumption and rising popularity with consumers and farmers makes it an important species for studies like this.

Proposed Work plan/Formulation and structure of study
Year Wise Plan of Research Work

First year:- Review of literature, collection of references, material and methods, designing of experiments, collection of samples will be done.

Second year:- Collection and preservation of samples, processing of samples, preparation of histological slides and biochemical analysis of tissues.

Third Year:- Analysis of statistical data, documentation and presentation of data, calculations, results, discussions, conclusion and summary. Preparation of synopsis and draft of thesis.

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